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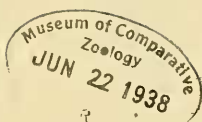
Parthenogenesis as illustrated in the late Dr. J. A. van Rossum's experiments with Pseudoclavellaria amerinae L. (Hym. Tenthr.)

by

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During the period 1897—1904 the late Dr. J. A. van Rossum made in Holland a number of experiments in parthenogenesis with the saw-fly *Pseudoclavellaria amerinae* L. (1), known to him as *Clavellaria amerinae* L. Some very interesting results were recorded from time to time (2) but the death of the experimenter unfortunately prevented their being collated and discussed in the manner they deserve. A close examination of the data shows that they are too few for the formulation of many definite conclusions but that they do suggest interesting possibilities, especially if considered along with my own extensive breeding and cytological work with other species, notably *Thrinax macula* Kl. Such a consideration, however, would not be appropriate for this journal and the principal object of this paper is firstly to state clearly and exactly what facts v. Rossum discovered, and secondly to indicate the interesting possibilities raised by these facts; incidentally, opportunity is taken to introduce the terms 'parthenogone' and 'parthenogonic' to replace respectively the more cumbersome expressions 'parthenogenetically-produced organism' and 'parthenogenetically-produced' respectively.*

THE RESULTS OF THE BREEDING EXPERIMENTS.

Parthenogenesis. These can be conveniently set out and understood by means of Tables 1, 2 and 3. One cardinal point that should be made clear is the type of offspring produced by individual parthenogenetic females. On this only three experiments furnish clear-cut information, one relating to the originator of the strain (1898), a second to a female

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* For these terms I am indebted to Dr. O. Skutsch and Mr. W. L. Lorimer, my colleagues of the Classical Department.

1897. Larva found (XLVI).
1898. *Female emerged April 28; produced 35 eggs from which were obtained 18 larvae (XLIII).

FIRST PARTHENOGONIC GENERATION

1900. 1 ♂ emerged April 13, after 2 years' hibernation (XLIII).

1899. 3 ♂ ♂ and 8 ♀ ♀ emerged April 11-May 14 (XLIII). *Six of these females laid 100 or so parthenogenetic eggs giving 55 larvae, of which 45 spun cocoons (XLIII).

1901. 1 ♂ and 1 ♀ emerged early in April, after 3 years' hibernation. 1 ♂ also found dead (XLIV).

SECOND PARTHENOGONIC GENERATION

1901. 8 ♂ ♂ and 2 ♀ ♀ emerged April 13-21, after 2 years' hibernation (XLIV, XLV).

1900. 13 ♂ ♂ and 2 ♀ ♀ emerged April 13-28 (XLIII, XLV). *One of these females laid about 60 eggs (XLIII, XLV), which developed parthenogenetically, giving 52 larvae. Only 27 larvae survived to spin cocoons (XLIV).

1902. 14 ♂ ♂ and 5 ♀ ♀ emerged April 12-May 15, after 3 years' hibernation. 2 ♂ ♂ also found dead in cocoon (XLV). *Three of these females produced 70 larvae (XLVI).

THIRD PARTHENOGONIC GENERATION

1902. 4 ♂ ♂ emerged April 18-May 3 after 2 years' hibernation (XLV).

1901. 13 ♂ ♂ emerged April 12-21, (XLIV).

1903 7 ♂ ♂ emerged March 22-27, after 3 years' hibernation (XLVI, p. 63).

1903. 7 ♂ ♂ and 3 ♂ ♂ emerged March 24-29 (XLVI, p. 63). *One of these females produced 19 larvae parthenogenetically (XLVI).

1904. 5 ♂ ♂ and 4 ♀ ♀ emerged April 12-30, after 2 years' hibernation (XLVII).

1905. 1 ♀ emerged May 4, after 3 years' hibernation (XLVIII)

FOURTH PARTHENOGONIC GENERATION

1904. 9 ♂ ♂ and 1 dead ♂ (XLVII, p. LV).
1905. 1 dead ♂ (XLVIII, p. LIX).

Table 1. The details of v. Rossum's experiments in parthenogensis with *Pseudoclavellaria amerinae* L.
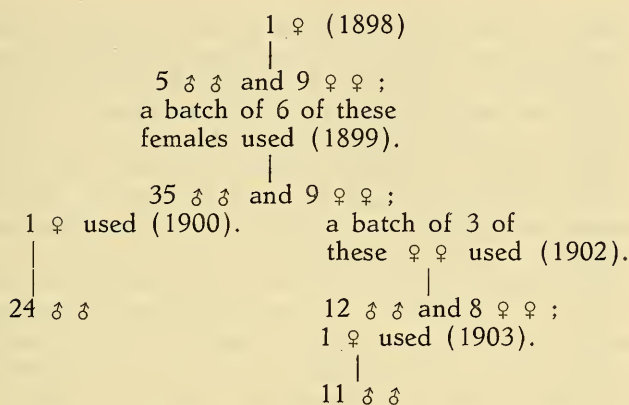* Denotes parthenogenetic breeding experiment. Roman figures refer to v. Rossum's records.

1 ♀ (1898)
|
5 ♂ ♂ and 9 ♀ ♀ ;
a batch of 6 of these
females used (1899).
|
35 ♂ ♂ and 9 ♀ ♀ ;

1 ♀ used (1900).   a batch of 3 of
| these ♀ ♀ used (1902).
| |
24 ♂ ♂   12 ♂ ♂ and 8 ♀ ♀ ;
                        1 ♀ used (1903).
                               |
                        11 ♂ ♂

Table 2. The sources and progeny of the parthenogenetic females

of the second parthenogonic generation (1900), and a third
to a female of the third parthenogonic generation (1903).
From the eggs of the first specimen 5 males and 9 females
were reared, from those of second specimen, 24 males, and
form those of the third, 11 males.

The first result suggests that some parthenogenetic females
are deuterotokous (amphoterotokous). But the deuterotoky is
of a special kind, which may be termed 'haploid-diploid',
because of the very different chromosome constitutions of
the two sexes produced. For in hymenoptera males are
haploid, being produced from unfertilised eggs which un-
dergo two maturation divisions and come to possess the
reduced or haploid number of chromosomes*, while partheno-
gonic females are diploid and arise from eggs in which the
diploid number is maintained or restored by some process of
autoregulation †; and this condition is to be distinguished
from that in certain other deuterotokously parthenogenetic
organisms where both male and female offspring are diploid.
The proportion of adults reared to eggs laid is 40% (Table
3), not a high figure, but the number and proportions of ma-
les and females obtained seem to rule out the possibility that

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* This statement is mainly based on researches in Tenthredinidae by
  Peacock and Sanderson (3), and Sanderson (4), which show that a
  number of tissues of the male have a chromosome constitution half that
  of the female. See also Schrader and Hughes-Schrader (5). The
  alternative view advanced is that the male has a diploid soma and a
  haploid germ-plasm ; see Vandel (6).
† Recent (unpublished) work on the thelytokous females of the saw-fly
  Thrinax macula Kl., by Peacock and Sanderson, shows that the egg
  undergoes one maturation division and remains diploid.
  In any case the two types of deuterotoky should be distinguished, for
  the two sexes in Hymenoptera are produced in different manners from
  those by which they are produced in certain other deuterotokous
  organisms.

either sex has been produced in an exceptional manner, as in such cases where occasional females are found in parthenogenetically arrhenotokous species (e.g., *Pteronidea ribesii* Scop.) or occasional males in parthenogenetically thelytokous species (e.g., *Pristiphora pallipes* Lep.).

The other two results, in which all-male broods were obtained, strongly suggest however that certain of the parthenogenetic females are purely arrhenotokous. The view is supported on other grounds to be discussed later.

In the specimens of 1900 the proportion of adults reared to eggs laid is also 40% ; the records of the specimens of 1903 do not permit an exact percentage being stated but it would certainly be lower than 58%, which is the approximate figure calculated from the proportion of adults obtained to the number of larvae which hatched.

The other breeding experiments in which both sexes occurred in the offspring give us no clue as to the parthenogenetic behaviour of individual females so that the reason or reasons for the deuterotoky manifested is unknown. The results obtained may have been due to one or a combination of the following phenomena : either individual parthenogenetic females were deuterotokous, as in the first exact result noted above ; or individual females might be either thelytokous — so far not known, or arrhenotokous, the latter condition being shown in the two exact results noted above.

Sex Ratio. In all, 12 females were used in five breeding experiments, particulars of which are given in the following table.

Date	Females	Eggs	Larvae	Offspring ♂	Offspring ♀	Percentage offspring : eggs
1898	1	35	18	5	9	ca 40
1899	6	100	55	35	9	ca 44
1900	1	60	52	24	—	ca 40
1902	3	—	70	12	8	less than 28.6*
1903	1	—	19	11	—	less than 57.9*

Table 3. Breeding results of five v. Rossum experiments.
* Calculated by reference to number of larvae obtained.

Efforts to arrive at some idea of the sex ratio from the figures of Table 3 are as follows :
(1) From all 12 females were obtained
                    87 ♂ ♂ and 26 ♀ ♀ (100 : 30)
(2) From the 3 isolated females were obtained
                    40 ♂ ♂ and 9 ♀ ♀ (100 : 22.5)
(3) From the two batches of 6 and 3 were obtained
                    47 ♂ ♂ and 17 ♀ ♀ (100 : 36)
(4) From the batch of 6 were obtained
                    35 ♂ ♂ and 9 ♀ ♀ (100 : 25.7)
(5) From the batch of 3 were obtained
                    12 ♂ ♂ and 8 ♀ ♀ (100 : 66.7)

Beyond recording these figures little more can be said. Provisionally the ratio of males to females may be regarded as being of the order 100 : 30, but it is obvious that until we know whether the individual parthenogenetic females are of two kinds, i.e.; deuterotokous and arrhenotokous, and until the breeding data from a sufficiently large number of individual females is known, a more precise ratio cannot be calculated. It will readily be seen, for instance, that the proportion of males derived from the batches of 6 and 3 in these experiments will be materially affected by the number of arrhenotokous females in each batch.

The Question of Differential Viability of the Sexes. Another factor which may operate in determining the sex ratio is differential sexual viability, and this factor is also to be considered in coming to any conclusion regarding the nature of the parthenogenesis shown by individual females.

The number of experiments performed and the proportions of adults reared (Table 3), judged by absolute standards, are not high. Nevertheless, according to my extensive experience with several other species, the experimental results are not without significance. That the females are less viable is not borne out by the 1898 experiment, for more females than males were obtained ; and that the males are the less viable is not shown by the 1900 and 1903 experiments, where males only were obtained. The experiments with batches of 6 and 3 females, while they show that the sex ratio is in favour of the male, do not however indicate a scarcity of females.

To my mind, the 1898 result, isolated though it is, should be interpreted as showing that some individual parthenogenetic females produce both males and females, and the 1900 and 1903 results as showing that other individual parthenogenetic females produce only males ; briefly, that the species, parthenogenetically, is both deuterotokous and arrhenotokous. The matter will be further discussed later. Further, it is considered that the sex ratio calculated from the experiments has not been influenced by differential sexual mortality but, rather, by the paucity of the experiments performed.

The Males, Sexual Behaviour, and Manner of Reproduction. Males are parthenogenetic products. It has been seen from 3 experiments that the males were derived from two types of parthenogenetic females, one deuterotokous and one arrhenotokous, while the other experiments with batches of parthenogenetic females do not allow us to draw any conclusions as to the manner of parthenogenetic male-production. The fundamental question of the determination of male-production will be dealt with presently but it may be said here that there seems no necessity for regarding the appearance of the male broods as being other than a phenomenon normal to the species.

Regarding the health and vigour of the males, v. Rossum notes that those of the second generation were no less lively than those of the first ; that those of 1901 belonging to the third generation were only very little smaller than those of the second, while those of 1903 belonging to the third generation, after three years' hibernation, were large ; that one of the fourth generation evinced readiness to pair. In my opinion there seems no season for the view that the vigour of males had diminished after four generations of parthenogenetic reproduction.

In pairing experiments three males and three females were used. The first two males emerged in 1902 after three years' hibernation but the type of mother which produced them (whether deuterotokous or arrhenotokous) is unknown. They showed no inclination to pair and v. Rossum mentions that this may have been due to the cold weather prevailing. The third male was derived from an arrhenotokous female of the third parthenogonic generation, and was a lively specimen which evinced readiness to pair. The female however resisted his efforts and a fight ensued in which she lost an antenna. The evidence is too scanty for arriving at any definite conclusion regarding the sexual behaviour of either sex, though many observations on the sexual behaviour of *Thrinax macula*, a species resembling in many respects *P. amerinae*, suggest the reasonable interpretation that the females carried on the strain without the assistance of the males and therefore did not need highly developed sexual instincts. The evidence from the three experiments of v. Rossum, small though it is, does not run counter to this explanation.

The origin of males from both deuterotokous and arrhenotokous females has been seen. How these conditions have arisen is a fundamental question that will be discussed later, though it may be said here that there is no necessity for regarding the appearance of the two male broods which terminated the experimental series as being due to a 'depression effect' of continuous parthenogenesis in the strain. It is true that the experiments came to an end because males only were produced, but, as only two females were used to carry on the strain (one in 1900 and the other in 1903), it is more likely that the strain died out because insufficient females were used to carry it on. Further, analogy with the conditions proved to exist in *Thrinax macula* suggests the reasonable interpretation that the appearance of the two male broods in *P. amerinae* was really a normal phenomenon, the males being the offspring of arrhenotokous females. It is of course possible that some environmental factor(s) could bring about a change in the manner of parthenogenetic reproduction, i.e., from deuterotoky to arrhenotoky, but the nature of such factors is entirely unknown.

One would not expect normal sexual behaviour in deu-terotokously parthenogenetic females, as in *P. amerinae,* or in thelytokously parthenogenetic females, as in *T. macula,* by the very fact of their having become able to produce by parthenogenesis. The change in their genetical constitution, whatever its physical basis, has possibly created a bar to fertilisation even when a male does succeed in pairing. That such might be the case in *P. amerinae* is suggested by my experiments with *T. macula* and *Pristiphora pallipes* in which the females show very little, if any, sexual behaviour, and in which infrequent pairings have been shown to produce no change in the kind of offspring normally produced in these species. Once female sexual instincts have become diminished or lost in this way two important consequences might follow : (1) certain of the males which the females may still be able to produce would be abnormal — undersexed ; 2) males, normal or otherwise, in the presence of the new type of females would not react sexually to the normal extent. The first idea is supported by current views of cytology and genetics into which we cannot enter here. The second idea is not proved, for we lack knowledge of the stimuli that determine pairing in saw-flies. The absence of sexual behaviour on the part of the two *amerinae* males might have been caused by the cold weather (as suggested by v. Rossum) but the above argument shows an alternative explanation. If, however, the active behaviour of the third male be characteristic of *amerinae* males we see that bar to pairing or fertilisation is due to the nature of the female.

It is possible, nevertheless, that arrhenotokously partheno-genetic females might still retain their sexual instincts and would pair, but no proof of this exists in v. Rossum's or any other saw-fly experiments.

SUGGESTED INTERPRETATION OF PARTHENO-GENESIS IN P. AMERINAE.

Bearing in mind how few are the known facts, it is neces-sary to proceed with caution in the attempt to interpret them. The following argument is therefore tentative. If we accept at their face-value the fact that there are two types of broods — one mixed and the other all-male, and the reasonable view that the species is maintained solely by the deuterotokous females, it follows that the deuterotokous females give rise the females of like kind, and, further, that there is the possi-bility of a second type of female, the arrhenotokous. The question then arises as to the origin of the arrhenotokous females, and the answer is : from the deuterotokous type, because there is no other source, for arrhenotokous females cannot produce their kind. The deuterotokous females must

then be capable of producing males, arrhenotokous females, and deuterotokous females.
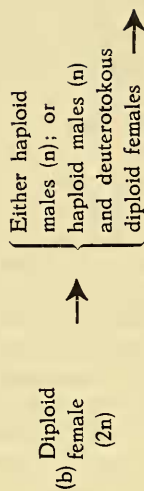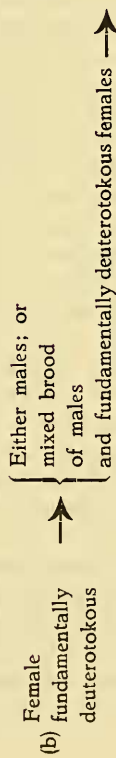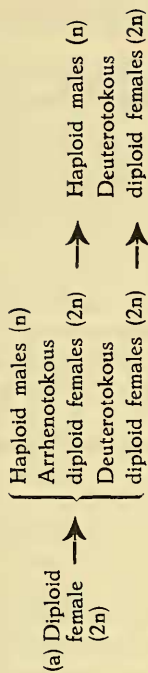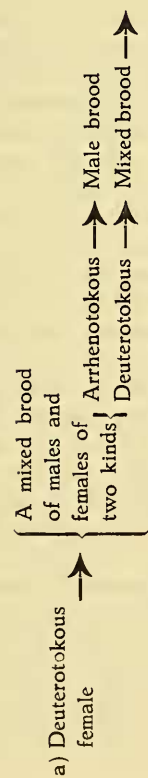
The next problem relates to the incidence of these different kinds of individuals. One way of accounting for them is that the *individual* deuterotokous female can produce a mixed brood composed of males and two kinds of females, one purely arrhenotokous and the other deuterotokous. This, however, raises the more fundamental question as to what factors influence the deuterotokous females to produce these three types of offspring. This may be attacked by considering it cytologically ; diploid deuterotokous females produce reduced, haploid, male-producing eggs, as well as autoregulating diploid eggs which will give rise to diploid male-producing females, together with autoregulating diploid eggs which will yield again the diploid deuterotokous type of female.

It is not very material to this paper as to what cytological processes produce these ends but what is important is that the eggs of one and the same female might be influenced to develop in three different ways. The influences at work may be hereditary or environmental, or both, but of these we possess no information. However, if the influences are hereditary, the course of events can be stated as above. (See Scheme below.) On the other hand, if we suppose the action of environmental factors, we are forced to suppose also that the reproductive material is unstable and can be affected to produce different kinds of eggs in different proportions. Once we suppose this there arises an alternative suggestion, namely, that, in reality, there is only one kind of female, which, under certain conditions, produces nothing but males, and, under others, a mixed brood of males and females. (See Scheme below.) In the first case the male *broods* are the grandchildren of a deuterotokous female, in the second the children. Yet, whichever is correct, the same principle is involved, namely, that the deuterotokous female is in an unstable state.

With the interpretation in mind that *P. amerinae* maintains itself by diploid parthenogenetic females but possesses to a certain extent the now useless faculty for arrhenotokous parth enogenesis, it is suggested that this species has been derived from a form (still the most common) which reproduced bisexually but was arrhenotokously parthenogenetic. How the faculty for deuterotokous parthenogenesis was acquired is unknown, presumably by some mutation. The reactions of this new faculty on the habits of the species are likewise unknown, though modern discoveries in cytology and genetics enable us to envisage some of them. These questions, however, lie beyond the scope of this paper. It would appear, however, that v. Rossum's experiments show that *amerinae* is a species in which there has occurred the

Biological

a) Deuterotokous female → A mixed brood of males and females of two kinds { Arrhenotokous → Male brood ; Deuterotokous → Mixed brood →

(b) Female fundamentally deuterotokous → { Either males; or mixed brood of males and fundamentally deuterotokous females →

Cytological

(a) Diploid female (2n) → { Haploid males (n) Arrhenotokous diploid females (2n) → Haploid males (n) Deuterotokous diploid females (2n) ; Deuterotokous diploid females (2n) →

(b) Diploid female (2n) → { Either haploid males (n); or haploid males (n) and deuterotokous diploid females →

Biological and cytological schemes illustrating interpretations of parthenogenesis in *Pseudoclavellaria amerinae*.

loss of the bisexual process and the establishment of a new manner of reproduction, by a new type of female, rendering arrhenotokous parthenogenesis useless.

SUGGESTIONS REGARDING THE EVOLUTION OF THE DIFFERENT KINDS OF PARTHENOGENESIS IN TENTHREDINIDAE.

My study of v. Rossum's work, in conjunction with my own work on other forms, leads me to suggest, for some species at least, the probable course of evolution of the different kinds of parthenogenesis in saw-flies. The primitive condition was that of normal bisexuality, a condition now unknown in the group. The next was the development of arrhenotokous parthenogenesis whereby males come to be produced by a peculiar method, the continuation of the species however still depending upon bisexual reproduction ; this is the condition which exists today in the majority of sawflies investigated and is probably found in all except those entirely dependent upon the females. The manner of evolution of this type of parthenogenesis has been ably studied by the Schraders (5). Later, deuterotokous parthenogenesis entered and it is to be expected that at first it co-existed with the arrhenotokous type ; no species showing these characteristics have been discovered and it may be that none now exist owing to the success of the new method of reproduction. Next would be the success of the deuterotokous type of female at the expense of both the male-producing female and sexual type of female, or, to put it another way, the success of continuous female parthenogenesis over bisexual reproduction ; under what circumstances such occurred is not known, though its occurrence is apparently testified by *P. amerinae.* The further stage would show the diminution of deuterotokous parthenogensis on the part of individual females and the substitution of thelytokous parthenogenesis, together with the diminution in number of males ; again the circumstances determining these are likewise unknown but their operation seems to be testified in *Thrinax macula,* where, although thelytokous parthenogenetic reproduction is the rule, an occasional female sporadically may produce either a male brood containing odd females or an all-male brood. A later stage is shown in such types as *Pristiphora pallipes* where thelytokous parthenogenetic reproduction is fixed, though a very small percentage of males occurs, these males being entirely useless in reproduction. It is of course possible that rare males are accidental products, the results of some unusual irregularity in the maturation of eggs normally thelytokous, in which case it may be that thelytoky has arisen directly at some stage prior to, and independent of, the evolution of deuterotoky. No indi-

cations of this exist and the known biological facts, e.g., those relating to *P. amerinae* and *Thrinax macula*, indicate rather that thelytokous parthenogenesis has supplanted the deuterotokous, and the latter the arrhenotokous, during the course of the evolution of the different kinds of parthenogenesis exhibited in the Tenthredinidae. Lastly comes the stage where only females exist, e.g., in *Allantus* (*Emphytus*) *pallipes* Lep. and others, this end being arrived at via deuterotoky, though, as just mentioned, some such forms may have developed thelytoky directly.

A detailed and critical consideration of these suggestions is impossible without the aid of cytology and genetics, subjects outside the scope of this paper, so it is proposed to reserve such consideration for a future communication.

ACKNOWLEDGMENTS.

SUMMARY.

1. The late Dr. J. A. v. Rossum's records relating to his experimental observations on parthenogenesis in the sawfly *Pseudoclavellaria amerinae* L. (Tenthredinidae), published between 1898—1905, have been collated in order to ascertain exactly what they reveal. The data proved to be too few for the formulating of many definite conclusions, though they suggest a number of interesting possibilities, especially when read in conjunction with the extended observations by the writer of this paper on the species *Thrinax macula* Kl., the habits of which in many ways resemble those of *P. amerinae*.

2. Beginning with a virgin female v. Rossum reared a strain in which four parthenogonic generations were obtained, the strain terminating when males only were obtained. The writer of this article does not regard this as showing that continuous parthenogenesis brought about male-production by some 'depression process'; it is more probable that the strain ended because insufficient females were used for breeding.

3. The number of experiments in parthenogenetic reproduction was small, five. Three separate virgin females were used : one gave a mixed brood of and 5 ♂ ♂ and 9 ♀ ♀, another 24 ♂ ♂, and another 11 ♂ ♂. It would appear therefore that both deuterotokous and arrhenotokous parthenogenesis exists in the species. In an experiment

involving a batch of six virgin females 35 ♂ ♂ and 9 ♀ ♀ were reared, and in another involving a batch of three virgin females 12 ♂ ♂ and 8 ♀ ♀ ; these experiments also show that deuterotokous parthenogenesis exists in the species but give no information regarding the parthenogenetic habit of *individual* females.

4. The five experiments, involving the use of 12 parthogenetic females, produced 87 ♂ ♂ and 26 ♀ ♀ and this ratio of 100 : 26 may be regarded provisionally as approximating to the sex ratio. Until the breeding results from a larger number of individual females are known an exact figure is impossible.

5. There is no indication of differential viability between the sexes.

6. Three pairing experiments, involving three males and three females, were unsuccessful ; but the reason for this is unknown. As, however, the breeding experiments show that the strain was carried on so far by females only, it seems likely that the species is maintained by females only, and that the development of this type of parthenogenesis has resulted in a diminution or loss of the sexual instincts of the females. How far the males are abnormal in sex behaviour is unknown.

7. The production of male broods by certain *amerinae* females parallels what occurs normally in the commonest type of saw-fly, where the unfertilised (but fertilisable) females are male-producing. It follows that these *amerinae* females may likewise be fertilisable, though there is no evidence of this. The arrhenotokous females must be the offspring of deuterotokous females for there is no other source for them.

   To account for the production of arrhenotokous parthenogenetic females and deuterotokous parthenogenetic females it is suggested that, in reality, there exists only one kind of female—deuterotokous, but reproductively unstable ; such a female, under certain conditions, produces a mixed brood of males, of male-producing females, and of deuterotokous females, but, under other conditions, produces *either* males only, or a mixed brood of males and of deuterotokous females. Whether the sex-determining conditions are inherent or/and environmental remains unknown.

8. The evolutionary aspect is discussed by the present writer. The arrhenotoky of *P. amerinae*, it is suggested, is vestigial, and the species is to be regarded as having originated by mutation from the form of saw-fly, still most common, where males are produced parthenogenetically from unfertilised eggs and females sexually from fertilised eggs. By considering the facts relating to other species it is further suggested that at least one course of evolution of

parthenogenesis in the Tenthredinidae has been as follows : primitively bisexually-reproducing forms have been succeeded by arrhenotokously parthenogenetic forms, which subsequently mutated to produce deuterotokously parthenogenetic forms, from which arose the partially then completely thelytokously parthenogenetic forms.

The detailed cytological and genetical consideration of parthenogensis in sawflies is reserved for a later paper.

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